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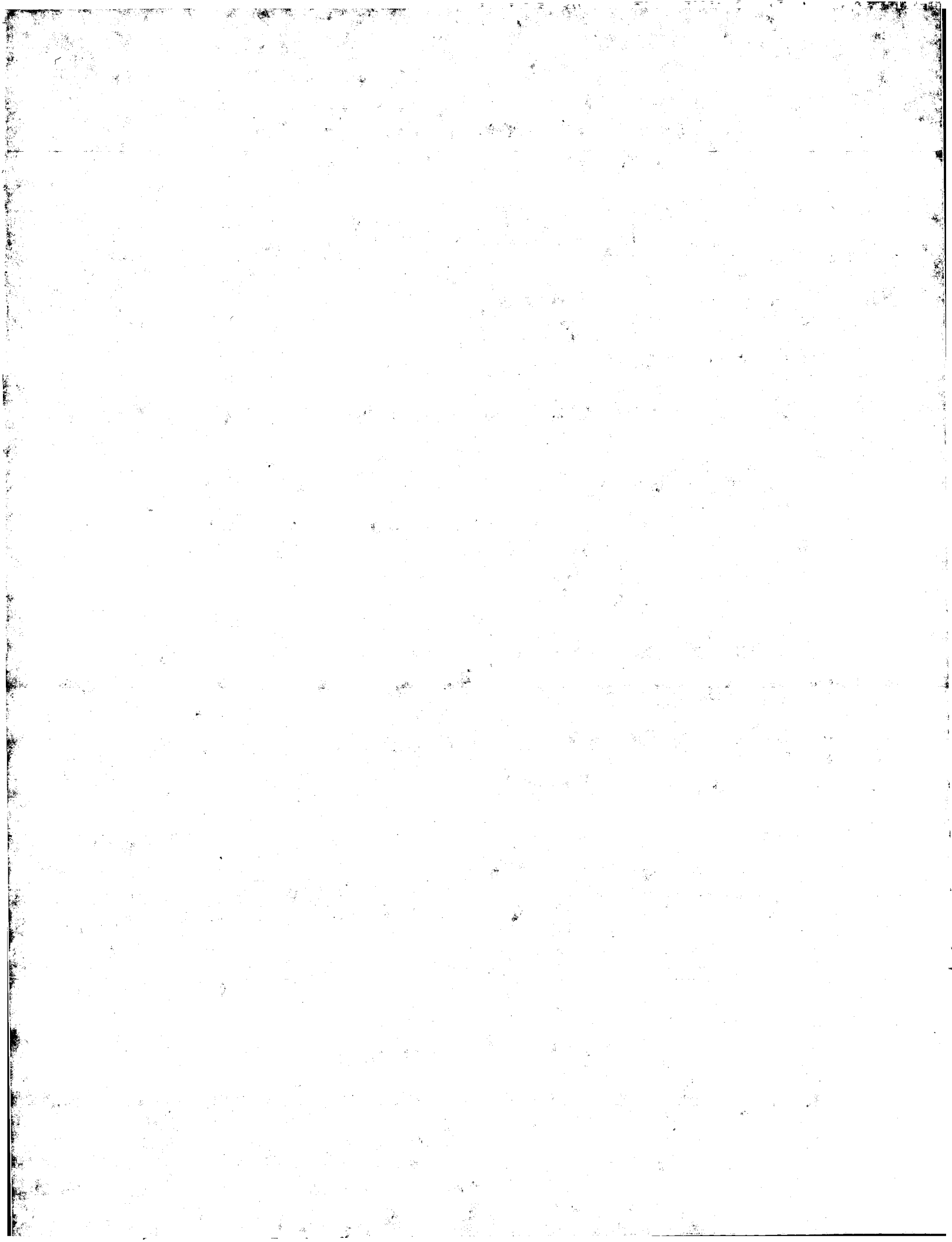
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WORLD INTELLECTUAL PROPERTY
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INTERNATIONAL APPLICATION PUBLISHED UNDER

WO 9606134A1

(51) International Patent Classification 6 : C08L 33/24, B01J 20/26, C02F 1/28	A1	(11) International Publication Number: WO 96/06134
		(43) International Publication Date: 29 February 1996 (29.02.96)

(21) International Application Number: PCT/US95/10636

(22) International Filing Date: 21 August 1995 (21.08.95)

(30) Priority Data:

08/292,889

19 August 1994 (19.08.94)

US

(71) Applicant: GEL SCIENCES, INC. [US/US]; 213 Burlington Road, Bedford, MA 01730 (US).

(72) Inventors: GOLD, Harris; 18 Peachtree Road, Lexington, MA 02173 (US). TANAKA, Toyochi; 22 Lowell Road, Wellesley, MA 02181 (US). ENGLISH, Anthony, E.; General Delivery, Roberts Creek, British Columbia V0N 2W0 (CA). KING, Kathleen, Rose; 39 Farm Road, Stow, MA 01775 (US). LEVY, Rhonda, Dulmage; 53 Ash Street, Hopkinton, MA 01748 (US). MASAMUNE, Satoru; 182 Brookline Street, Newton, MA 02159 (US). WANG, Changnan; Apartment 135, 402 Ringe Avenue, Cambridge, MA 02140 (US).

(74) Agent: JIMENEZ, Mary, Raynor; Choate, Hall & Stewart, 53 State Street, Exchange Place, Boston, MA 02109 (US).

(81) Designated States: AU, CA, CN, FI, JP, KR, MX, NO, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published*With international search report.**Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.*

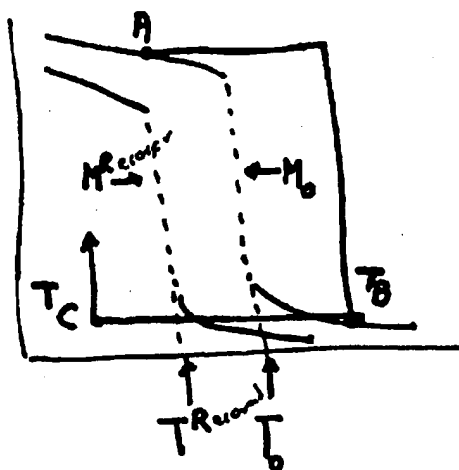
(54) Title: RESPONSIVE GELS FOR SELECTIVE REMOVAL OF A TARGET FROM AN ENVIRONMENT AND METHODS THEREFOR

(57) Abstract

Responsive polymer gels that selectively remove a target from an environment are described. A three-dimensional, reversibly responsive polymer gel comprises at least two binding moieties for the target, capable of binding the target when they come into proximity to each other to form a binding site. The binding moieties are combined with a responsive polymer component that is adapted to undergo reversible volumetric collapse and expansion in response to a change in an environmental condition. The polymer gel component has a phase-transition threshold for volumetric collapse different from the phase-transition threshold for volumetric expansion. The responsive gel is constructed so that as the polymer gel collapses to a collapsed position at a

phase-transition threshold, the binding moieties are placed in sufficient proximity to each other and to the target to allow the binding site to bind the target. As the polymer expands to an expanded position at a phase-transition threshold, the binding moieties are disengaged from each other. Method of removing a target from an environment is also described and includes the steps of contacting the polymer gel of the invention with the target, the target having binding affinity for the binding site and allowing the gel to move to a collapsed position, so that the binding moieties come into sufficient proximity to each other and to the target to bind the target at the binding site. Methods of selectively removing a chosen target from a solution of the target and another target are also described. In preferred embodiments, the step of altering an environmental condition comprises altering temperature of the gel.

Volume



Environmental Condition

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Responsive Gels for Selective Removal of a Target from an Environment and Methods Therefor

This invention described and claimed herein was supported, in part, by Department of Energy (DOE) contract DE-FG02-94ER81667 awarded to Foster Miller, Inc. The U.S. Government has certain rights to this invention.

Background of the Invention

Selective absorption and recovery of target molecules are among the most fundamental processes in biological systems as well as in chemical and medical industries. Nevertheless, in contrast to naturally-occurring biological systems, most artificial systems that purport to allow for target recovery lack selectivity for the target and the ability for reversible release of the incorporated target. In many cases, recovery of target molecules requires extensive desorption and washing with a solvent, which creates serious environmental problems.

As but one example of the myriad of procedures used to selectively recover a target molecule from an environment containing the target, we briefly discuss heavy metal pollution in water and wastewater. Although some metals are essential dietary constituents in trace quantities, many are toxic at higher concentrations and attack the nervous system and kidney function. There are a number of processes that are commonly used to treat metal-bearing waters and wastewaters. The most conventional treatment technology is precipitation by itself or in conjunction with other treatment processes that enhance its efficiency. Precipitation involves the adjustment of solution pH by chemical addition to cause a supersaturated solution and the resultant precipitation. Precipitation is non-selective and generates a residue (sludge) requiring disposal. It is very difficult to separate and recover metals from precipitates for subsequent refining. The precipitate has low chemical purity and because of its gelatinous nature, phase separation is very difficult. Precipitation also has the problem that metals are concentrated from dilute waters at the expense of adding chemicals, although innocuous, beyond the stoichiometric requirements to increase the total quantity of materials required for disposal.

Other technologies that can be used to recover metals from waters include evaporation, ion exchange, membrane separation, and electrowinning. Evaporation involves the concentration of contaminated waters by evaporation of the water. The main advantage of evaporation is that the metal concentrate can be reused directly at the proper concentration. The major disadvantage of evaporation is that it is an energy intensive process whose cost depends on the volume of water that must be evaporated. Ion exchange is a well-established process for metal removal and recovery that dates back to the 1950's. Ion exchange has been used successfully in the metal finishing industry for the purification of spent plating and processing baths for reuse and the production of deionized water. The primary disadvantages of ion exchange relate to the fact that ion exchangers must be regenerated for continual reuse, otherwise the process would be prohibitively expensive. However, the process is still relatively expensive compared to precipitation, because of the cost of the regenerant chemicals. As in precipitation, the total quantity of sludge is increased as a result of the addition of the chemical regenerants beyond the stoichiometric requirements. Another disadvantage of this process is that most commercial ion exchangers are generally non-selective and recovery of specific metals is difficult. There are some commercially-available ion exchangers that are selective to specific metals. However, the process requires much larger quantities of regenerant chemicals.

**Table 1. Processes for Treating
Metal-Contaminated Waters**

PROCESS	METAL RECOVERY	DISADVANTAGES
Precipitation	No	Non-selective Residue requires disposal Precipitate has low chemical purity Phase separation is difficult Chemicals added to increase material disposal

Evaporation	Yes	Energy intensive High operating and capital costs Secondary contaminants are concentrated
Ion Exchange	Yes	High operating costs Non-selective Chemicals added for regeneration - additional sludge generated
Membrane	Yes	High operating and capital costs Non-selective Membranes susceptible to fouling and deterioration Metal recovery difficult
Electrowinning	Yes	High operating costs Limited to relatively concentrated solutions

Electrodialysis and reverse osmosis membranes are commercially available for pollution control and resource recovery. They are not extensively used for metal recovery because they have both high capital and operating costs, the membranes are susceptible to fouling and deterioration, the membranes are non-selective, and metal recovery is difficult to achieve because of the inability to economically achieve sufficiently high brine concentrations. Electrowinning involves the application of an electrical potential to plate out and recover metals. The principal applications have been the recovery of cadmium from plating rinsewaters and copper from spent printed circuit board baths. Process inefficiencies and associated economics limit applications to relatively concentrated solutions.

In summary, most processes used to treat metal-bearing waters have one or more of the following disadvantages: high operating costs, non-selective metal removal; additional sludge is generated beyond the stoichiometric requirements and must be disposed of because of the use of treatment chemicals.

Ricka and Tanaka, "Phase Transition in Ionic Gels Induced by Copper Complexation," *Macromolecules*, 18, 83-85, 1985, developed gels that were copolymers of acrylamide and acrylic acid. The gels underwent swelling in solutions of target (copper ⁺² ions), and the swelling increased with increasing

acrylic acid content, indicating that the acrylic acid of the gels was absorbing copper ions as the gel expanded in volume. At higher Cu^{+2} concentrations, this dependence was reversed and the gels shrank with increasing acrylic acid content. The stimulus for the volumetric responsiveness of the gel is the concentration of the target (copper) in solution. This gel system is not practical because the stimulus for regenerating the gel, i.e., bringing the gel back to the collapsed state where it can bind to the target, is the solution itself. Since the present gels and methods are effectively employed when the target concentration is relatively fixed, the Ricka/Tanaka gel cannot be used since the target concentration has to be varied to induce the volumetric response. The acrylamide-acrylic acid gel used by Ricka and Tanaka is not suitable for heavy metal removal from groundwaters at higher concentrations, say, 0.1-1 M, not only for the reasons enumerated above, but because it cannot be regenerated with a low cost stimulus.

Summary of the Invention

The present invention pertains to responsive polymer gels that have unique properties, enabling them to be used to selectively remove a target from an environment. One embodiment of a gel of the invention is a three dimensional, reversible responsive polymer gel comprising at least two binding moieties for the target, the binding moieties capable of binding the target when they come into proximity to each other to form a binding site. The binding moieties are combined with a gel that is a responsive polymer component adapted to undergo reversible volumetric collapse and expansion in response to a change in an environmental condition. The polymer gel component has a phase-transition threshold for volumetric collapse that is different than the phase-transition threshold for volumetric expansion. The responsive gel is constructed so that as the polymer gel collapses to a collapsed position at a phase-transition threshold, the binding moieties are placed in sufficient proximity to each other and to the target to allow the binding site to bind the target. As the polymer expands to an expanded position at a phase-transition threshold, the binding components are disengaged from each other and from the target. Most preferably, the two phase-transition thresholds differ from each other because of differences in target concentration and because of differences in kind of target.

The responsive polymer component undergoes a volumetric change driven primarily by interactions that include ionic bonding, hydrophobic bonding, hydrogen bonding and van der Waals bonding and an exemplary responsive polymer component is N-isopropylacrylamide, and an exemplary binding moiety is acrylic acid. The most preferred polymer gels of the invention are reversibly responsive to a change in temperature of the gel and the gel has at least two different phase-transition temperature thresholds.

A method of removing a target from an environment include the steps of contacting the polymer gel of the invention with the target, the target having binding affinity for the binding site and allowing the gel to move to a collapsed position, so that the individual binding moieties come into sufficient proximity to each other and to the target to bind the target at the binding site. A further method for removing a target from an environment containing the target, includes the steps of contacting the polymer gel of the invention with the target to be recovered and forming a binding site in the gel by altering the temperature of the gel to induce a volumetric collapse thereof so that target is bound in the gel. Once bound, the temperature of the gel is again altered to induce a volumetric expansion of the gel. The gel is regenerated by releasing target from the gel. Preferably, the steps of altering the temperature and regenerating occur substantially at the same time. The most preferred means for regenerating the gel is contacting the gel with an elution agent.

Gels of the invention may also selectively remove a chosen target from a solution of the target and another target. The method steps include contacting the polymer gel of the invention with the target and another target in which the gel has a lower phase-transition threshold in the presence of the target than in the presence of the other target. Next, the environmental conditions of the gel are altered to a condition that is intermediate the phase transition thresholds of the two targets. The gel is induced to move to a collapsed position, whereby the binding moieties come into sufficient proximity to each other and to the target to bind the target having the lower of the two phase-transition thresholds. In preferred embodiments, the step of altering an environmental condition comprises altering temperature of the gel.

The responsive gels described herein have the potential to overcome many

of the problems associated with conventional methods used to remove targets from an environment of use. They have an ability to significantly reduce energy consumption because of their unique characteristics that include: (i) an ability to be regenerated with stimuli that itself do not produce wastes, such as heat and light; (ii) an ability to be regenerated with low cost stimuli; (iii) an ability to be regenerated with small changes in the external stimulus; (iv) reversible properties; (v) an ability to remove different targets over a wide range of target concentration; (vi) an ability to remove target from solutions and reduce the target solution concentration, while producing a highly concentrated target solution upon regeneration of the gels; (vii) an ability to selectively remove one target in preference to another; (viii) an ability to remove target from solutions having a broad spectrum of innocuous substances, such as other inorganic salts; and (ix) inexpensive to manufacture.

Brief Description of the Figures

Figure 1 is a schematic illustration of target binding and elution by a gel having two phase-transition thresholds, one at the original concentration of the target ($M = M_0$), and one at the higher, elution concentration (M^{recovery}).

Figure 2 is a graph of the degree of swelling of a NIPA/Acc (500mM/200mM) copolymer gel as a function of copper $^{+2}$ ion.

Figure 3 is a graph of the degree of swelling of a NIPA/Acc (500mM/200mM) copolymer gel as a function of kind of metal ion.

Figure 4 is a graph of the degree of swelling of a NIPA/AAm/Acc (684mM/8mM/8mM) copolymer gel as a function of 1 mM calcium and copper ions.

Figure 5 illustrates the swelling response of a NIPA/Acc (500mM/200mM) copolymer gel when the target concentration is 10 micromolar copper or calcium.

Figure 6 shows the degree of swelling of a NIPA/Acc (500mM/200mM) copolymer gel as a function of three different divalent metal ions (Cu^{+2} , Ca^{+2} , and Fe^{+2}) at the same concentration.

Figure 7 shows the degree of swelling of a copolymer gel made of NIPA (500mM) and AAc (200 mM) in 0, 10 μM and 15 mM copper ion.

Figure 8 shows the degree of swelling of a NIPA-AAc gel as a function of

target concentration at 35 °C.

Figure 9 shows the degree of swelling of a NIPA-AAc gel as a function of target concentration and at 40 °C.

Figure 10 shows the degree of swelling of a NIPA-AAc-AAm gel (188 mM/256 mM/256 mM) as a function of temperature for various target concentrations (0 μ M, 6.8 μ M, and 50 μ M).

Detailed Description of the Invention

Gel Systems

GENERAL CONSIDERATIONS:

The polymer gels of the present invention are molecular machines that preferably contain two groups of monomer building block components that are combined, although a single monomer building block with dual characteristics is also contemplated. One building block component may be ionized and is a target binding moiety. The other building block component allows the polymer containing the target binding moieties to undergo a reversible volumetric collapse and expansion, as described in more detail below.

A significant feature of the present invention is that both components work in concert with each other. The target binding moiety is combined with, and distributed throughout, the polymer as this monomer component is polymerized and incorporated into the gel. When at least two target binding moieties are attached to positions within an expanded gel, it is considered that the gel is capable of forming at least one "binding site". At least two target binding moieties are required to bind the target.

That is, the target will most preferably bind to the binding site only when the gel is collapsed so that the two binding moieties and the target come in close enough proximity to each other, just as two finger tips must come closer to pick up an object. Selection of the monomeric binding moieties allows precise control of polymer affinity and selectivity to targets. In a sense, the gel consists of "muscle" and target recognizing "tweezers". The "tweezers" can capture a target when the "muscle" closes the "tweezers" and release the target when the "muscle" opens them.

The proximity of the binding moieties to each other and to the target, and

therefore the formation of the binding site of the gel, can be controlled by the volumetrically responsive building block component. The gel can be physically expanded and contracted between two end points, e.g., a collapsed position and an expanded position. Thus, when the gel is in the collapsed position the binding sites are formed and are able to bind to target molecules, but when the gel is in its expanded position the binding affinity of the binding sites is weakened because the two or more binding moieties are physically separated and the target may be released from binding. This transformation is preferably reversible. The advantage of this configuration is that the binding affinity can be weakened with small changes in an external environmental condition, releasing the target and allowing the polymer gel to be recycled for use.

The present gels are materials between the liquid and solid state, consisting of a cross-linked network of long polymer molecules. The term "gel" refers to a three-dimensional, crosslinked polymer network that includes a liquid solvent entrained by the interconnected matrix of polymer chains. The term "polymer network" refers to polymers crosslinked to create a three-dimensional, tangled network. The term "gel" more particularly refers to polymer networks between the liquid and solid state containing enough solvent molecules to cause macroscopic changes in the sample dimension. The term is also meant to include gels in their "dry" condition, in which substantially all solvent that is within the gel matrix has been removed. The term is primarily an operational definition. One definition of the term is when the mass of the gel reaches a constant low value in desiccator or drying oven.

The preferred gels are "reversibly responsive", i.e., when challenged with an environmental change, the environmental change affects the gel by causing the entire gel, or a component thereof, to undergo a reversible volumetric change. It is preferred that the gel undergo a reversible volumetric change of at least 20 percent in response to a change in an environmental condition, in which the gel expands from a less liquid-filled state or dry state to a more liquid-filled state; or collapses from a more liquid-filled state to a less liquid-filled state. The reversible volume change involves a shift between two equilibrium positions (i.e., swollen and collapsed).

The gels may be fabricated in a variety of forms, such as microporous gels.

The term "microporous" refers to two-phase systems of a continuous solid phase containing numerous pores filled with fluid. A "microstructure" as defined herein, refers to those structures of a gel (e.g., pores, voids, walls and the like) observable under a scanning electron, or other, microscope and ranging in size from 0.01 to about 100 microns. Gels containing pores in the size range 0.01 to about 10 microns are 'microporous'. If some of the pores are interconnected, the gel is typically called an "open-cell" gel. If all the pores in the gel are interconnected to each other, the gel is a "bicontinuous" gel. If the pores are discrete (not connected to each other), so that the internal space of each pore is independent of the other pores, the gel is a "closed-cell" gel. The present invention encompasses as all these morphological forms and combinations of these forms.

The reversible volume change of the entire gel, or a component thereof, may be either continuous or discontinuous. A "continuous" volume change is marked by a reversible change in volume (i.e. a collapse or swelling) that occurs over a relatively large change in environmental condition. Moreover, there exists at least one stable volume near the transition between the swollen and collapsed positions.

Gels of the invention may undergo a "discontinuous" volume change in which the reversible transition from swollen to collapsed positions, and back again, occurs over an extremely small change in environmental condition, such as less than 0.1 degree C or 0.1 pH unit. Such reversible gels are hereinafter called "phase-transition" gels and the small increment in environmental condition at which the volumetric change takes place is called the "phase transition threshold" (e.g., phase transition temperature threshold; phase transition pH threshold ; phase transition light energy threshold and the like). See Tanaka et al. U.S. Patent 4,732,930, or Hirotsu et al., J. Chem. Phys. 87: 15 July 1987 describing synthetic polymeric gels that undergo phase transitions, incorporated herein by reference. There is no stable volume between the swollen and collapsed positions at the phase-transition and, in theory, the expansion and/or collapse occurs over an infinitely small environmental change. A gel undergoing a continuous phase-transition may have a similar order of magnitude total volume change as a gel undergoing a discontinuous phase-transition. Responsive phenomena have been

observed in many permanently crosslinked polymer networks that exist as gels. Tanaka, Physical Review Letters, Vol. 40, no. 12, pp. 820-823, 1978 and Tanaka et al, Physical Review Letters, Vol. 38, No. 14, pp 771-774, 1977; Tanaka et al Physical Review Letters 5, Vol 45, pg. 1636, 1980; Ilavsky, Macromolecules, Vol. 15, pg. 782, 1982; Hrouz et al, Europ. Polym. J., Vol. 17, pg. 361, 1981; Ohmine et al, J. Chem. Physics, Vol. 8, pg. 6379, 1984; Tanaka et al, Science, Vol. 218, pg. 462, 1982 and Ilavsky et al, Polym. Bull. Vol. 7, pg. 107, 1982; Gehrke, "Responsive Gels: Volume Transitions II"; ed. K. Dusek, Springer-Verlag, New York, pp. 81-144 (1993); Li et al., Ann. Rev. Mat. Sci., 22: 243-277 (1992); and Yu et al., Enzyme Microb. Technol., 15: 354-366 (1993), all of which incorporated herein by reference.

On a molecular level, the preferred responsive gels are sensitive to small changes in a restricted repertoire of environmental "trigger" conditions consisting primarily of temperature, pH, light, and solvent concentration. On a macroscopic level, any of a variety of environmental conditions may be imposed on the gel which allows the specific trigger to induce a volume change. These environmental conditions may, but not necessarily, be the same as the trigger and include, but are not limited to, a change in temperature, electric field, photon energy, pH, solvent composition, concentration of biomolecules, pressure, and the like. The responsive gels of the invention may be combined with a material that acts as a molecular "transducer", converting an environmental condition into an appropriate trigger. For example, a dye may be introduced into a temperature-responsive gel. The dye is designed to absorb light of a given energy and convert the light energy into heat, thus triggering the gel to undergo a temperature-induced volumetric change at a phase-transition threshold. See also, A. Suzuki and T. Tanaka, Nature: 346: 6282 (1990), incorporated herein by reference.

As mentioned above, the significant disadvantage of the prior art responsive gels of Ricka and Tanaka is that fact that their phase-transition thresholds will occur as a function of target concentration. The most preferred gels of the present invention are designed to have phase-transitions triggered by easily generated and easily controlled environmental factors such as temperature or light.

Moreover, the methods of the invention, described below, rely in part on other characteristics of the gels that have not been heretofore appreciated as being

significant in this context; namely a shift in phase-transition threshold as target concentration is changed and a shift in phase-transition threshold with different targets at the same target concentration. We have observed that the phase-transition threshold of temperature responsive gels will be shifted towards a lower temperature as the target concentration increases (see Example 2 and Figure 2). In particular, if the target is a multivalent ion, it is believed that the target's binding affinity will provide an additional attractive force between the individual polymers of a collapsed gel. Theoretical considerations, see T. Tanaka, D.J. Fillmore, S-T. Sun, I. Nihio, G.A. Swilslow, and A. Shar, Phys. Rev. Letters, 45 1636 (1980) and U.S. Patent 5,100,933 (Tanaka et al.), incorporated herein by reference, suggest that this added attractive force will lower the phase-transition threshold of the collapsed gel. The phase-transition threshold of light, pH and solvent-responsive gels may also be similarly affected by target concentration. Using the gel synthesis procedures described herein and the general principles known for developing gels responsive to a variety of environmental triggers, persons having ordinary skill in the art may routinely determine if the phase-transition threshold of a given gel is influenced by the target concentration.

Another significant characteristic of the present gels is that, at a constant target concentration, the phase-transition threshold of temperature responsive gels will be shifted towards a lower temperature as the kind of target is varied (see Figures 3 and 4). The binding affinity of a given size and type of target to the target binding moieties of the gel is primarily determined by the nature of the chemical bonds developed between target and binding site. This effects the attractive forces developed between polymers of the gel and will therefore also influence the phase-transition threshold.

I. POLYMER STRUCTURE

A. Responsive Component

The primary requirement of a gel of the invention is that the entire gel, or its reversible volume change component, undergo a reversible volume change. The gel as a whole must meet these requirements. Nevertheless, the gel includes a binding moiety as long as at least one component(s) provides the required volume response property.

For instance, the gel of the invention may be a single material such as a single polymer network which meets the volume response and target binding site requirements. Most preferably, however, the gel include two or more components, each component having the different required property. For example, a copolymer gel may be fabricated in which one component has is capable of forming at least one binding site that has a binding affinity with a target; the other component having the volume change property that is responsive to a change in an environmental condition.

A primarily binding-type monomer may be polymerized in the presence of a volume-change gel. Exemplary gels of this type include poly-N isopropylacrylamide [NIPA: "responsive component"]-poly(acrylic acid) ["binding moiety"].

The gel may also be an interpenetrating polymer network (IPN). An IPN may possess a volumetric response property such as poly-N isopropylacrylamide and it may be combined with a binding moiety such as acrylic acid to meet the requirements of the present system. The IPN may possess both properties so that one polymer member of the IPN provides the binding property and the other polymer member provides the responsive property. Polymers of an interpenetrating gel to be loaded can include natural polymers, synthetic polymers, or crosslinked natural and synthetic polymers.

The most preferred volume response components may consist, in whole or in part, of polymers made by copolymerization/crosslinking of monofunctional and polyfunctional polymerizable vinyl monomers. Exemplary gels may contain N-alkylacrylamide (or analogous N-alkylmethacrylamide) derivatives like N-ethylacrylamide, N-n-propylacrylamide, N-n-propylmethylacrylamide, N-isopropylacrylamide, N-n-isopropylmethylacrylamide, N-cyclopropylacrylamide, or acrylate (or analogous methacrylate) copolymers like hydroxypropyl acrylate-co-acrylamide, diacetone acrylamide-co-hydroxyethyl acrylate, hydroxypropyl acrylate-co-hydroxyethyl acrylate, ethylacrylamide, cyclopropylacrylamide, n-propylacrylamide, and isopropylacrylamide.

Reversible volume change components of the invention may also be made by crosslinking linear polymers through physical interactions as in the poly(vinyl

alcohol)-poly(acrylic acid) or poly(ethylene glycol)- poly(methacrylic acid) systems, in which these hydrophobically modified polyethylene glycol and similar polymers can associate through strong hydrophobic interactions. Charge complexation and hydrogen bonding also works well, particularly for pH-sensitive gels. Examples are poly(ethylene glycol)- poly(methacrylic acid) or poly(vinyl alcohol)-poly(acrylic acid).

Also preferred are natural polymeric starting materials that may be chemically cross-linked. Exemplary polymers that may be conveniently used according to the invention include precursors such as alkyl-substituted cellulose derivatives like cellulose ethers. Exemplary cellulose ethers include methylcellulose, hydroxyethylcellulose, hydroxypropylmethylcellulose, hydroxypropylcellulose, carboxymethylcellulose and hydroxymethylcellulose. Polypeptides like poly(L-proline), and poly(valine-proline-glycine-X-glycine), [where X - tyrosine, phenylalanine, leucine, valine, glutamic acid, lysine, glycine, and other amino acids] may also be used. Exemplary polysaccharides include starches, sugars, chitin, and hyaluronic acid.

The volumetric changes of gels described herein result from competition between intermolecular forces, usually electrostatic in nature, that act to expand the polymer network; and at least one attractive force that acts to shrink it.

Volumetric changes in the gels of the invention are driven primarily by four fundamental forces acting between the polymer chains themselves and between the polymer chains and the solvent. The forces include: ionic, hydrophobic, hydrogen bonding and van der Waals bonding interactions, either alone or in combination. Each of these interactions may be independently responsible for a volume change in preferred gels of the invention. Each of these fundamental forces is most strongly affected by a particular trigger. Changes in solvent concentration most strongly affect the van der Waals interaction; changes in temperature most strongly affect hydrophobic interactions and hydrogen bonding; and changes in pH most strongly affect ionic interactions.

Thus, a responsive gel whose volume change is governed by ionic interactions would include as its constituents weakly acidic and weakly basic building blocks, such as poly(acrylic acid)/poly(methacrylamidopropyltrimethylammonium chloride[MAPTAC])/water;

poly(acrylic acid)/poly(allylamide)/water, and the like. See, Siegel and Firestone, Macromolecules, 21: 3254-3259 (1988). Gels of this type are sensitive to pH and will collapse when exposed to a lower pH environment from a higher pH environment.

Responsive gels whose volume change is governed by hydrogen bonding will collapse with a decrease in temperature (i.e., expand at a higher temperature) and are exemplified by interpenetrating polymers that comprise poly(acrylic acid) as one polymer, poly(acrylamide) as the other polymer, and water as the liquid medium or copolymers of dimethacrylamide and methacrylic acid. Gels whose volume change is governed by hydrophobic interactions will collapse when challenged with an increase in temperature (i.e., expand at a lower temperature) and are exemplified by poly(N-isopropylacrylamide:NIPA). See U.S. Patent 4,863,613. Moreover, NIPA may be combined with a molecular "transducer" such as protoporphyrine or chlorophylline to render the gel light sensitive. Gels whose volume change is governed by van der Waals interactions will display temperature responsiveness similar to those gels governed by hydrogen bonding and are exemplified by polyacrylamide gels.

Responsive gels may be formulated in which the volume change is governed by more than one fundamental force. In particular, gels consisting of copolymers of positively and negatively charged groups meet this requirement. In these gels, polymer segments interact with each other through ionic interactions and hydrogen bonding. The combination of these forces results in the existence of several pH-driven phases. See Annaka and Tanaka, Nature 355: 430-432 (1992), incorporated herein by reference. Equations qualitatively explain all of these aspects of volumetric changes. See T. Tanaka, D.J. Fillmore, S-T. Sun, I. Nihio, G.A. Swilslow, and A. Shar, Phys. Rev. Letters, 45 1636 (1980) and U.S. Patent 5,100,933 (Tanaka et al.), incorporated herein by reference. See also, S.H. Gehrke, Adv. Polymer Science 110:81-144 (1993), for other theoretical descriptions.

The mole percentage of the responsive component of the gel may vary over a wide range but not every combination of components will yield gels having the characteristics described herein. There is a specific ratio of responsive component/binding moiety, balanced between having too much responsive

component and not enough binding component, that will be suitable for a given application. Preferred ranges for the responsive component are between about 400 mM to about 5M, while usually no more than 5M binding moiety is suitable. No more than routine experimentation is required to synthesize gels having a range of component values.

B. Binding Component

There is an extensive database in the field of selective ion exchange in which various affinity groups have been identified for selected targets. Nevertheless, if one were to indiscriminately use a given affinity group as the target binding monomer component for the gels of the present invention, the resulting gel would merely act like a selective ion exchanger regardless of whether the gel was in the expanded or collapsed positions. In this case, each binding site would be a single monomer binding component and volumetric expansion and/or contraction of the gel will have no effect on the affinity of the single binding component for the target. However, if the binding component were to be synthesized in such a way so as to effectively split the binding component into at least two moieties (i.e., iminodiacetic acid would be split into two moieties, each having an acetic acid group), so that at least two moieties are required for binding, then the collapse and expansion of the gel would dramatically effect the binding affinity of the gel for the target, a criteria that distinguishes the present gels from those of the prior art. Thus, it is not obvious from merely knowing target/affinity binding pairs that a given binding component is appropriate for the present gels. Using routine experimentation, those of ordinary skill in the art may readily split a particular binding component into two moieties (or chemically synthesize the moieties *de novo*), polymerize them to form a responsive gel, and perform the necessary collapse and expansion of the gel to determine if the binding site is reconstituted and active when the gel is collapsed.

Binding moieties preferably include portions of: (i) alpha-amino acids with functional side chains, such as portions of glutamic acid, lysine, ornithine, aspartic acid, cystine, histidine, tyrosine and p-aminophenylalanine; (ii) di-aminodicarboxylic acids such as diaminopimelic acid; (iii) iminodiacetic acid and its derivatives; (iv) anthranilic acid and its derivatives; (v) salicylic acid and its

derivatives; and (vi) diethylenetriamine. Binding moieties specific for lead, zinc and copper may preferably include portions of : (i) ethylenediaminetetraacetic acid (EDTA) because of its flexibility in complexing a wide range of metals, including Cu, Zn and Pb; (ii) pyrogallol, for its affinity for lead ; and (iii) picolyl amines because of their effectiveness at low pH. See Calmon, ("Specific Ion Exchangers," in *Ion Exchange for Pollution Control* (Calmon, C. and Gold, H., eds.), Vol. II, 151-154, CRC Press, Boca Raton, FL, 1979; and " Specific and Chelate Exchangers: New Functional Polymers for Water and Wastewater Treatment," *J.A.W.W.A.*, 73, 652-656, 1981, incorporated herein by reference,

Examples of preferred target binding moieties (and their collapsed, binding site forms) include carboxylic acids (polycarboxylic acids), iminodiacetic acid (EDTA), isothiuronium (polyisothiuronium), oximes (e.g., dimethylglyoxime), and picrylamine (dipicrylamine). Most preferably, binding moieties will not bind target when the gel is expanded, but will bind target when the gel is collapsed.

Although the preferred binding moieties will not bind target in the expanded gel position, it will be understood that, in certain embodiments, individual binding moieties may actually provide some target binding in the expanded gel position, albeit with a binding affinity much weaker than when the binding moieties are in the collapsed position. The most common example is iminodiacetic acid which, when reconstituted as a binding site, yields EDTA. By itself, iminodiacetic weakly binds divalent metal ions, but once collapsed the resulting EDTA will very strongly bind specific divalent ions.

The binding moieties are generally copolymerized and cross-linked to the responsive gel matrix during the gel polymerization. Most preferably, if the responsive polymer component is NIPA, the binding monomer is synthesized with vinyl groups to enable them to attach to the NIPA matrix.

Binding moieties are introduced preferably in a quantity of about 1 - 100 moles per every hundred monomer units in the polymer, more preferably about 2 - 20 moles per hundred monomer units.

Biologically significant molecules may also be separated using the gels and methods described herein. For example, fragments of the variable regions of light and heavy immunoglobulin chains may be included as the binding moieties of the present gels. Production of antibodies and various fragments thereof are well

within the level of skill of those in the art and methods of immobilizing these materials into polymers are also well known. The term "antibodies" is meant to include monoclonal antibodies, polyclonal antibodies and antibodies prepared by recombinant nucleic acid techniques that are selectively reactive with a target. The term "selectively reactive" refers to those antibodies that react with one or more antigenic determinants of a target and do not react with other targets. Antigenic determinants usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and have specific three dimensional structural characteristics as well as specific charge characteristics. By way of example, antibodies may be raised against amino-terminal (N-terminal) or carboxy-terminal (C-terminal) residues of a given target peptide by methods that include: (i) immunizing an animal with the target that is expressed by a prokaryotic (e.g., bacterial) or eukaryotic cell; the cell including the nucleotide coding sequence for all or part of the target peptide; or (ii) immunizing an animal with whole cells that are expressing all or a part of the peptide. To further improve the likelihood of producing an antibody towards a given peptide target, the amino acid sequence of peptide targets may be analyzed in order to identify portions of target which may be associated with increased immunogenicity. For example, peptide target sequences may be subjected to computer analysis to identify potentially immunogenic surface epitopes. Such computer analysis can include generating plots of antigenic index, hydrophilicity, structural features such as amphophilic helices or amphophilic sheets and the like. For preparation of monoclonal antibody binding moieties directed toward biologically relevant molecules, any technique that provides for the production of antibody molecules by continuous cell lines may be used. For example, the hybridoma technique originally developed by Kohler and Milstein (Nature, 256: 495-497, 1973), as well as the trioma technique, the human B-cell hybridoma technique (Kozbor et al., Immunology Today, 4:72), and the EBV-hybridoma technique to produce human monoclonal antibodies, and the like. See also Larrick et al., U.S. Patent 5,001,065 and (Ladner et al. U.S. patents 4,704,694 and 4,976,778) and references cited therein.

Those having ordinary skill may readily ascertain other design rules needed to provide the present gels with appropriate binding moieties for a given target.

By way of example only, the coordination chemistry of metal ions is well-established and it is understood that the copper ion has six ligand sites to be filled by six groups that provide electrons such as, for example, four oxygens of a carboxyl group and two nitrogens of an amine group. See, for example, D.D. Ebbing, General Chemistry (ed. M.S. Wrightson), Houghton Mifflin Co., Boston, 1984, incorporated herein by reference.

Anions may also be suitable targets. In this case, suitable binding moieties may include amines, pyridines and amadine (See Calmon, *supra*).

Conventional methods for cross-linking monomer/polymer starting materials may be used to synthesize the gels of the present invention with routine experimentation from starting materials which are known are which are conventionally prepared. Polymerization is initiated using a polymerization initiator, e.g., a free radical initiator such as ammonium persulfate, sodium metabisulfite, or azobisisobutyronitrile, with dilution with a solvent, e.g., water, a lower alcohol, hydrocarbon, etc., or without dilution. However, neither the solvent nor the polymerization initiator are always important factors to obtain the polymerized product from the monomer mixture, and any method suitably selected from conventionally well-known gelation methods may be applied. Crosslinking can also be induced by ultraviolet or electron beam irradiation.

Polymers of the invention may also be affixed onto a matrix or membrane. For example, the materials may be used in support matrices, films or membranes, tubes, hollow fibers, solid fibers, molded objects, solid particles, capsules, micelles or liposome-like structures. In particular, preparation of *N*-isopropylacrylamide responsive gel beads has been reported by emulsion or suspension polymerization. See, Hirose *et al. Macromolecules* 20, 1342-4 (1987); U.S. Patent No. 5,183,879 to Yuasa *et al.*; and JP 90-260558 to Kohjin Co., Ltd., all of which are incorporated herein by reference. In a typical suspension crosslinking process, an aqueous polymer suspension is introduced into a continuous organic phase. The polymer suspension must be immiscible in the continuous organic phase. When the polymer is present in the aqueous phase and introduced into a continuous organic phase, as is the case for the cellulose ether polymers of the present invention, the process is known as "inverse suspension crosslinking". The aqueous phase containing the polymer is agitated or stirred in

the continuous organic phase, thereby dispersing the aqueous phase as droplets in the continuous organic phase. A crosslinker is included in the suspension which crosslinks the polymer. The crosslinker is preferably a bi functional or multi functional chemical compound which is capable of reaction with the polymer in the droplet across two or more sites on the polymer chains, thus forming a polymer network which retains the droplet size and shape. The crosslinker is typically added to the aqueous suspension prior to dispersion into the organic phase, although it may be added during or after dispersion of the aqueous phase into the continuous organic phase. In addition, surfactants may be added to the continuous organic phase to stabilize droplet formation and control droplet size. Defoaming agents may be added to the aqueous solution to promote droplet formation and avoid foaming.

C. Cross-linking Agents

The additional requirement for the practice of this invention is that the gel be crosslinkable, preferably chemically cross-linkable. Any reagent which can react with two or more groups on the polymer can function as a crosslinker and convert that polymer to a gel. Polymers with reactive side groups like hydroxyl, amide, or carboxyl will be among the easiest to crosslink (note that these groups are also water soluble groups). It is thus most convenient if the crosslinker is water-soluble.

Suitable cross-linkers include acetaldehyde, formaldehyde, N,N' - methylene-bis acrylamide, ethylene glycol dimethacrylate, glycerine triacrylate or divinylbenzene or the like, glutaraldehyde, diglycidyl ether, divinyl sulfone, diisocyanates, epichlorohydrin, phosphoryl chloride, trimetaphosphate, trimethylmelamine, polyacrolein, and ceric ion redox systems. The concentration of crosslinkable material is generally about 0.1 to about 10 mole percent based upon the polymerizable material which is the main component. The crosslinking agent effects partial crosslinking of the polymer and provides a means to control the gel's mechanical strength, swelling degree, and intensity of volume change trigger by changing the crosslinking density. Preferred crosslinkers for polysaccharide volumetric response components, especially cellulose ethers, are multifunctional carboxylic acids, such as adipic acid (hexanedioic acid:

HOOC(CH₂)₄COOH), succinic acid (HOOC(CH₂)₂COOH), malonic acid (propanedioic acid: CH₂(COOH)₂), sebacic acid (decanedioic acid: HOOC(CH₂)₉COOH), glutaric acid (pentanedioic acid: HOOC(CH₂)₃COOH), or 1,10 decanedicarboxylic acid. Dicarboxylic hydroxyacids such as tartaric acid and malic acid as well as multifunctional carboxylic acids such as 1,2,3,4-butanetetracarboxylic acid may also be suitable. Unsaturated dibasic acids have been used to physically crosslink water soluble polymers by application of drying and/or heat. See, for example, U.S. Patent 3,379,720 (Reid, incorporated herein by reference.

D. Catalysts

Polymerization initiators, such as a free radical initiator such as ammonium persulfate or sodium metabisulfite, are usually not required in the present methods. Catalysts may, however, be required such as hydroxide that will catalyse reactions with polyvinylsulfone.

II. METHODS OF SELECTIVELY REMOVING A TARGET

A. General Considerations:

The procedure generally consists of causing a gel that lacks bound target to go to a collapsed position which (i) brings the binding monomer groups into proximity to form binding sites; (ii) increases the strength of the affinity between the target and the binding sites; and (iii) desolvates the gel. Then the gel is regenerated. This is accomplished by placed in its expanded position to reduce the strength of the affinity binding. An eluting agent is then added to displace the target metal ions from the active sites and replace the target in the gel with an appropriate ion from the elution solution.

To demonstrate the principles of the invention, a copolymer gel consisting of binding monomer (acrylic acid (CH₂=CHCOOH = Acc) and N-isopropylacrylamide (CH₂=CHCONHCH(CH₃)₂ = NIPA) was synthesized. See Example 1. Pure NIPA gel undergoes a reversible volume change at 33°C in water when the temperature is varied. Carboxyl groups of at least two acrylic acid monomers can form a binding site with one divalent cation.

When target copper ions are added as a solution to the gel, the

concentration of copper ions inside the expanded polymer was the same as outside the polymer. When the gel was induced to collapse, the concentration of target metal ions in the supernatant solution decreased, indicating that binding sites only appear when the gel is in the collapsed position. The supernatant solution was separated from the gel and the gel was induced to expand in deionized water. There was very little release of target from the gel.

In another experiment, the supernatant solution was separated from the gel and the gel was induced to expand in hydrochloric acid. The concentration of target in the acid was about 3-4 times the initial concentration of target in the acid. These experiments suggest the following principles: (i) the binding sites disappeared in both cases when the gel attained its expanded position but the target ions were still weakly attached to the expanded gel because of the requirement of overall charge neutrality of the gel; (ii) in order to displace the target metal ions, H^+ ions or other positive ions must be substituted for the target metal ions. Deionized water has a very low concentration (10^{-3} M) of H^+ and will only displace the target metal from the binding moieties of the gel over a very long time period. Hydrochloric acid will, however, contribute to the ionic displacement with sufficient speed because of its higher H^+ concentration. Conversely, binding of anionic targets requires elution with bases such as NaOH to provide hydroxyl anions.

In another experiment, as target metal ions were added, the phase transition temperature threshold was incrementally decreased due to extra intrapolymeric attractions created by target metal ions. The magnitude of the shift increased with the target concentration. The shift of the phase transition temperature threshold with changes in target concentration can be exploited in operation of molecular target recovery.

The process of recovering a target from solution involves two basic operations:

(i) removal of targets from the solution and their affinity binding into the collapsed gel matrix; (ii) elution (removal) and recovery of the target from the gel. The principles of removing and recovering a target from solution was successfully demonstrated with particular regard to heavy metal removal and are summarized below and schematically illustrated in Figure 1, plotting volumetric change versus

environmental trigger (e.g., temperature, light, pH). See also Examples 1 and 2.

The method of operating the process for any given target depends on (a) the chemical and physical properties of the gel, (b) the affinity binding of the target from solution onto the gel matrix, and (c) elution and recovery of the target from the gel matrix.

1. Sorption

An exemplary sorption process for heavy metal target is as follows: (See Figure 1): Assume a solution of target copper ions of concentration M_o and we wish to concentrate and recover the copper ions in a higher concentration $M^{recovery}$. Suppose that a first phase transition threshold condition is T_o at M_o and a second threshold is $T^{recovery}$ at $M^{recovery}$, where $T^{recovery} < T_o$, due to the depression of phase-transition threshold with increasing target concentration.

First, a swollen gel is placed in the original solution (M_o) at one environmental condition T_A ($< T_o$); and $T^{recovery}$ less than or equal to T_A . Copper ions freely diffuse in and out of the gel. The condition is altered to T_B (i.e., higher temperature, more light, higher pH: where $T^{recovery} < T_B$ and $T_B > T_o$). The gel undergoes a volumetric collapse and active sites are formed by the proximity of binding moieties so that the gel is loaded with copper ions. Solvent is disgorged from the collapsing gel and the supernatant solution outside the collapsed gel becomes dilute and can be discarded or recovered as a purer solvent.

2. Elution/Regeneration

An eluting solution is then contacted with the collapsed gel so that the target solution concentration, after the gel is expanded, will become $M^{recovery}$ (See Figure 1). The condition is then altered (i.e, temperature lowered, light levels lowered, pH lowered) to T_C $<$ or equal to $T_A < T^{recovery} < T_o$. The gel undergoes a volumetric expansion. Bound copper ions are eluted and released in the concentration $M^{recovery} > M_o$. The eluting solution contains a cation (i.e., HCl, NaCl) and is chosen so that the cation (hydrogen or sodium ions) will replace all of the metal ions within the gel. The gel is now expanded and wet without much metal ions in it and is ready to go into the next cycle as a regenerated gel. The second cycle starts by placing the gel in the original solution. Although Figure 1

illustrates a gel collapsing with an increase in the magnitude of an environmental condition, gels may be synthesized that show the reverse trend and the target extraction process may be altered accordingly without departing from the scope of the invention.

The selectivity or the difference in the affinity to target molecules is manifested in the difference in the phase transition threshold. See Example 3 and Figure 3. We have demonstrated that phase-transition threshold temperatures are almost the same for the divalent cations Ca, Co, Mg, and Mn. Substantial shifts are observed for divalent cations Zn, Cu, Fe, and Pb. Selective absorption and recovery can be achieved by making use of the difference in the phase transition temperature thresholds between different targets. For example, suppose we want to separate Cu^{+2} ions from Ca^{+2} ions. By choosing the temperature for gel collapse intermediate between the phase transition threshold temperatures of calcium and copper ($T_{\text{Cu}} < T_A < T_{\text{Ca}}$), more Cu^{+2} ions are accumulated within the collapsed gel.

Such selection was indeed achieved by a NIPA/Acc/AAM (684mN/8mM/8mM) gel 684 immersed in a solution of Cu^{+2} and Ca^{+2} ions, each having the same concentration (1 mM) at 40°C. See Figure 4. In the concentration range where the gel was collapsed there was a marked selectivity: The gel absorbed more than five times as many Cu^{+2} ions than Ca^{+2} ions. The selectivity was destroyed when the gel was swollen.

Figure 5 illustrates the swelling response of a NIPA/AAC (500 mM: 200 mM) gel when the target is 10 micromolar. Phase transitions are now discontinuous (arrows denote the volume change where there is no apparent intermediate volume condition between the expanded and collapsed states: compare closed triangles: calcium ions and closed squares: copper ions). Figure 5 also illustrates the effect of target concentration on phase-transition thresholds. Compare the phase-transition temperature threshold at the collapse of the gel in copper ions, in which the copper concentration of the gel is increased (closed squares: downward arrow) to the expansion of the same gel at the new, higher copper concentration (open squares: upward arrow) .

The volumetric responses are drastically dependent on the species of metal ions. In experiments to determine the phase transition characteristics of the gels,

three different divalent metal ions were studied: Cu^{+2} , Ca^{+2} , and Fe^{+2} (Figure 6). The fact that they are different allows us to discriminate between these ions. The gel appears to be least selective to calcium. These respective ions can be selectively collected by choosing the proper temperatures for gel collapse and expansion.

Example 1: Responsive Gel Properties

The gel system consists of a co-polymer of N-isopropylacrylamide (NIPA), acrylic acid (AAc), and acrylamide (AAm). The polymer gel consists mainly of two groups, one responsible for volume phase transition, namely, NIPA, and the other responsible for selectively capturing the metal ions, namely AAc and AAm. The addition of the ionic monomers (AAc and AAm) makes the inherently non-ionic NIPA gel sensitive to pH, electric fields and solution electrolytes.

The total concentration of both groups is fixed at 700 mM, i.e., $[\text{NIPA}] + [\text{AAc}] + [\text{AAm}] = 700 \text{ mM}$. These components are cross-linked with 8.6 mM of N,N'-methylene bisacrylamide (BIS) and dissolved in deionized, distilled water using the standard method of free radical copolymerization. Polymerization (gelation) occurs at room temperature (20 °C) and is initiated by adding 1.76 mM of ammonium persulfate (APS) and accelerated by adding 8.6 mM of tetramethylethylenediamine (TEMED). After gelation is completed, the gel is immersed in large amounts of water to wash away the residual chemicals. The wet gel is then squeezed through a 450 μm mesh screen.

This NIPA-based gel has the following properties: (i) at room temperature the gel is in its expanded state; (ii) as the temperature is increased above its phase transition temperature threshold, the gel collapses, expelling any liquid in its matrix (the phase transition temperature for a gel in distilled water without any ionic monomers ($[\text{AAc}] = [\text{AAm}] = 0$) is approximately 34 °C); (iii) as the ionic monomers are added to the NIPA, the gel becomes ionized and the phase transition temperature threshold increases beyond 34 °C when the gel is exposed to distilled water; and (iv) when the gel is put into a metal-containing solution, the phase transition temperature threshold is reduced below that corresponding to distilled water and the phase transition temperature threshold shift increases monotonically with metal concentration.

The advantages of these gels are that the negative charge of the gel is permanently attached to the gel matrix and the diffusion time of metals into the pore structure is relatively fast, of the order of seconds for micron-size particles. In the NIPA-AAc-AAm gel system, the AAc and AAm act as the binding moieties and are covalently bonded to the NIPA matrix.

The experimental configuration for determining gel swelling or shrinking as a function of the stimulus that is used to trigger the response of the gel is generally a test tube which contains glass micropipettes of diameter d_0 (110 μm or 140 μm in diameter). After a pre-gel solution is added to the micropipette, gelation is completed inside the pipettes. The glass at one end of the pipette is cut and removed from around the gel. The pipette is suspended inside a larger tube in which the environment is controlled. For example, if the gel is sensitive to temperature, the pipettes are placed inside a temperature controlled bath and the temperature varied. The diameter of the gel that is exposed to the stimulus is measured optically with a microscope and recorded as a function of temperature. To determine the effect of copper concentration on the gel diameter, a copper solution having a constant concentration (and temperature) is allowed to flow over the gel and the equilibrium gel diameter, d , is measured. The temperature is then changed and the measurements are again made. In some cases, the temperature is continually increased and continually decreased to confirm that the behavior of the gels is reversible.

Table 1 shows the composition of gels whose swelling curves were determined.

Table 1. Gel Compositions

NIPA (mM)	AAc (mM)	AAM (mM)
700	0	0
684	8	8
668	16	16
572	64	64
444	128	128
188	256	256
672	128	0
500	200	0
450	250	0

These gels show different swelling curves and except for concentrations of AAc below 64 mM, all gels show significant changes in the phase transition temperature threshold as a function of target concentration. Figures 2 and 7 show the diameter of the copolymer gel made of NIPA (500mM) and AAc (200 mM) in five different copper ion concentrations: 0 , 1 μ M, 3 μ M, 10 μ M and 15 mM. There is a drastic decrease in the phase transition threshold with increasing copper ion concentration and this demonstrates the feasibility of removing copper ions from solution using these gels. The volumetric response becomes continuous at higher target concentrations (15 mM).

Figures 8 and 9 show the degree of swelling of a NIPA-AAc gel as a function of target concentration. The temperature is fixed at 35 °C in Figure 8 and at 40 °C in Figure 9. At the higher temperature (Figure 9), the transition becomes discontinuous. Figure 10 shows the degree of swelling of a NIPA-AAc-AAM gel (188 mM/256 mM/256 mM) as a function of temperature for various target concentrations (0 μ M, 6.8 μ M, and 50 μ M) and indicates a discontinuous transition only for distilled water.

Example 2: Gel Sorption

Batch experiments are carried out in the following manner. Approximately 10 grams of wet gel (containing distilled water), containing NIPA-AAc (500mM:200mM) is placed in a beaker that contained 50 mL of a copper chloride solution of various concentrations. The dry weight of the gel is 0.1 grams. The solution and gel are continuously stirred. The solution is heated to 45 to 50 °C, a few degrees above the phase transition temperature of the gel, and the gel collapsed. The metal concentration of the supernatant is then measured with atomic absorption spectrophotometry. The supernatant is drained and a little more than 20 mL elution agent (sodium chloride) at a temperature below the phase transition temperature is added to the gel. The gel expands and releases the copper ions. The copper concentration in the supernatant is measured.

Test results showing the sorption of metals from solution are shown in Table 2.

Table 2. Test Results - Sorption

Metal Solutions	C_{init} (meq/L)	Measured C_{init} (ppm)	Measured C_{exp} (ppm)	Removal (percent)
Individual				
Copper	0.63	21.0	5.5	69
Lead	0.63	67.0	9.9	83
Zinc	0.63	23.0	16.0	17
iron	0.63	19.0	0	100
Mixed				
Copper	0.31	10.5	0.12	99
Sodium	0.31			
Mixed				
Copper	0.16	5.4	1.53	66
Zinc	0.16	9.0	7.0	7
Lead	0.16	18.0	2.5	83
Iron	0.16	4.6	1.0	74
Mixed				
Copper	0.31	10.8	3.31	63
Zinc	0.31	14.0	11.0	6

Mixed				
Copper	0.31	9.8	1.79	78
Iron	0.31	8.5	1.67	76
Mixed				
Copper	0.31	10.0	3.12	63
Lead	0.31	31.0	4.5	83
Individual				
Copper*	3.4	109	15	73
* Wet gel = 30 gr; solution volume = 30 mL				

The percentage removal is the total amount of metal removed from the solution by the gels divided by the amount of metal found initially in the solution taking into account that the initial metal concentration is diluted by the water initially in the gel.

Table 2 shows that except for zinc, the gel removes more metal than is found in the pore volume of the gel indicating that the AAc is actually binding the target metals in solution. Based on the removal efficiency, the lead appears to be the most bound by AAc, followed by copper or iron; zinc does not appear to be strongly bound by AAc. The presence of sodium in the feed solution does not inhibit the uptake of the gel for copper. For a given gel, binding may be limited by the target concentration in the initial solution. If the target concentration is already too high in the feed solution, the gel may not have the capacity to bind more gel. There is an optimum ratio between the gel capacity and the total amount of ions in solution that will give the best recovery.

Example 3: Gel Elution and Regeneration

Initially, 0.1 mM sodium salt solutions were used to elute the gels of the target ions that were bound by collapse of the gel. Less than 10% of metal in the gel was eluted off. Hydrochloric acid was then used to elute the gels. The test results are presented in Table 3.

Table 3. Test Results - Elution

Metal Solutions	C _{init} (meq/L)	Measured Eluant (ppm)	Metal Bound by Gel (mg)	Metal Recovered (mg)
Individual				
Copper	21.0	63.0	0.72	0.63
Lead	67.0	250	2.76	2.50
Zinc	23.0	20.0	0.19	0.20
iron	19.0	1.7	0.95	0.016
Mixed				
Copper	10.5	38.0	0.52	0.38
Sodium				
Mixed				
Copper	5.4	18.9	0.18	0.19
Zinc	9.0	9.0	0.03	0.09
Lead	18.0	63.0	0.75	0.63
Iron	4.6	7.4	0.17	0.07
Mixed				
Copper	10.8	35.7	0.34	0.35
Zinc	14.0	14.0	0.04	0.14
Mixed				
Copper	9.8	27.4	0.38	0.27
Iron	8.5	12.9	0.32	0.13
Mixed				
Copper	10.0	24.1	0.31	0.24
Lead	31.0	90.0	1.28	0.90
Individual				
Copper*	109	195	4.55	1.95
* Wet gel = 30 gr; solution volume = 30 mL				

In general, most of the metal bound onto the gel matrix was recovered upon elution with HCl and the concentration of the metals in the wash solution was about three times higher than in the feed solution.

Utilities/Applications

Applications of this technology include pollution control, especially for metal ion removal and recovery/concentration of valuable metal ions from wastewaters and process solutions, such as metal plating baths. Ionic forms of precious metals may be separated conveniently from their salts or other metal compounds using the present gels and methods. The metals include gold, silver, platinum, palladium, iridium, osmium, rhodium and ruthenium. The term "salts" is intended to include soluble salts (including their solvated simple and complex ions) and insoluble salts and esters of the metals and the term "compounds" is intended to include the soluble and insoluble oxides, and combinations of metals and nonmetals, e.g., gold telluride and silver arsenide. Typical precious metal simple and/or complex ions are silver nitratoplatinite, diaminesilver perhenate, platinum monohydroxy chloric acid, tetramine platinum (II), chloride hydrate, nitratopentamine iridium nitrate, auric chloride, silver nitrate, and zinc tetramineperhenate. The ions to be treated include ores, industrial wastes and concentrates obtained via water purification or ore processing. For example, the ionic forms can be from the mining of free metals, metal salts or metal compounds; from jewelry manufacture; plating solutions; photographic film manufacture and processing; and from heavy metal contaminant cleanup. Typical salts from which metal anions are derived include the fluoantimonite, fluotitanate, fluogermanate of ammonia; metavanadate of potassium, and the cobaltinitrite, cyanocuprate, ferrate, metagermanate heptahydrate, iron oxalate, molybdate, tungstate, and silicotungstate dodecahydrate of sodium. The metal anion feed can result from refining, free metals, metal salts or metal compounds; from manufacturing processes, plating solutions; and from heavy metal contaminant clean-up.

Other applications include: water softening in which calcium is removed from hard water and replaced with either sodium or hydrogen; removal of negatively charged ions from solution, such as nitrates (originating from fertilizer)

found in drinking water sources; pollution control; and bioseparations in pharmaceutical and recombinant DNA manufacturing processes, especially the purification of biological products from moderate to dilute broths; purification is very highly selective for the product and removes impurities of similar chemical functionality and physical properties.

Equivalents

It should be understood that various changes and modifications of the preferred embodiments may be made within the scope of the invention. Thus it is intended that all matter contained in the above description be interpreted in an illustrative and not limited sense.

CLAIMS

1. A three dimensional, reversible responsive polymer gel comprising:
(i) at least two binding moieties for a target, the at least two binding moieties capable of binding the target when the at least two binding moieties come into proximity to each other to form a binding site; the at least two binding moieties combined with a (ii) a responsive polymer component that is adapted to undergo reversible, volumetric collapse and expansion in response to a change in an environmental condition, the polymer gel having a phase-transition threshold for collapse that is different than the phase-transition threshold for expansion, the responsive gel constructed so that as the polymer gel collapses to a collapsed position at a phase-transition threshold, the at least two binding moieties are in sufficient proximity to each other and to the target to allow the binding site to bind the target and, as the polymer expands to an expanded position at a phase-transition threshold, the at least two binding moieties are disengaged from each other and from the target.
2. The reversibly responsive polymer gel of claim 1, wherein the phase-transition threshold for expansion and contraction are different from each other because of a difference in target concentration.
3. The reversibly responsive polymer gel of claim 1, wherein, at a constant target concentration, the phase-transition threshold for expansion and contraction are different from each other because of a difference in kind of target.
4. The reversibly responsive polymer gel of claim 3, wherein the gel is in a collapsed position and includes a target bound to the collapsed gel.
5. The reversibly responsive polymer gel of claim 1, wherein the responsive polymer component undergoes a volumetric change driven primarily by an interaction selected from the group consisting of ionic bonding, hydrophobic bonding, hydrogen bonding and van der Waals bonding.
6. The reversibly responsive polymer gel of claim 1, wherein the responsive

polymer component is N-isopropylacrylamide.

7. The reversibly responsive polymer gel of claim 1, wherein each of the at least two binding moieties are the same and are selected from the group consisting of oximes, acrylic acid, iminodiacetic acid and derivatives thereof, salicylic acid and derivatives thereof, picolyl amines, isothiuronium, anthranilic acid and derivatives thereof, diethylenetriamine, and picrylamine.
8. The reversibly responsive polymer gel of claim 1, wherein each of the at least two binding moieties are the same and are selected from the group consisting of amines, pyridines and amadine.
9. The reversibly responsive polymer gel of claim 1, wherein each of the at least two binding moieties are the same and comprise a fragment of an alpha-amino acid.
10. The reversibly responsive polymer gel of claim 9, wherein the alpha-amino acid is selected from the group consisting of glutamic acid, lysine, ornithine, aspartic acid, cystine, histidine, tyrosine and p-aminophenylalanine.
11. The reversibly responsive polymer gel of claim 6, wherein the responsive polymer and the binding moieties are in a molar weight ratio of about 2.5 to 1.
12. The reversibly responsive polymer gel of claim 3, wherein the gel is reversibly responsive to a change in temperature of the gel and has at two different phase-transition temperature thresholds.
13. A method of removing a target from an environment, comprising:
 - contacting the reversibly responsive polymer gel of claim 3 with the target, the target having binding affinity for the binding site; and
 - allowing the gel to move to a collapsed position, whereby the at least two binding moieties come into sufficient proximity to each other and to the target to form a binding site that binds the target.

14. A method for removing a target from an environment containing the target, comprising:

contacting the reversibly responsive polymer gel of claim 3 with the target to be recovered;

forming a binding site in the gel by altering the temperature of the gel to induce a volumetric collapse thereof;

altering the temperature of the gel to induce a volumetric expansion of the gel; and regenerating the gel by releasing target from the gel.

15. The method of claim 14, wherein the steps of altering the temperature and regenerating occur substantially at the same time.

16. The method of claim 14, wherein the step of regenerating the gel comprises contacting the gel with an elution agent to displace target from the expanded gel.

17. A method for selectively removing a chosen target from a solution of the target and another target, comprising:

contacting the reversibly responsive polymer gel of claim 3 with the target and another target, wherein the gel has a lower phase-transition threshold in the target than in said another target;

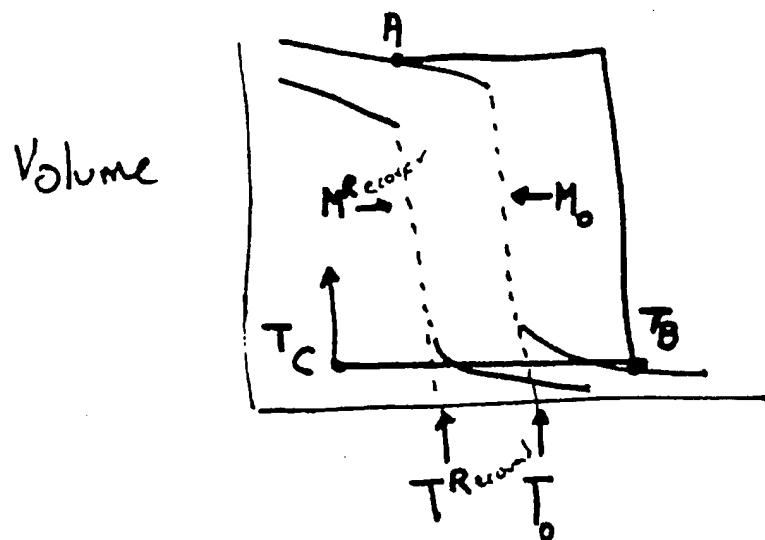
altering the environmental condition of the gel to a condition that is intermediate the phase transition thresholds of the target and said another target;

allowing the gel to move to a collapsed position, whereby the at least two binding moieties come into sufficient proximity to each other and to the target to bind the target having the lower of the two phase-transition thresholds.

18. The method of claim 17, wherein the step of contacting comprises contacting the gel with two different divalent cation targets.

19. The method of claim 17, wherein the step of altering an environmental condition comprises altering temperature of the gel.

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Environmental Condition
Figure 1

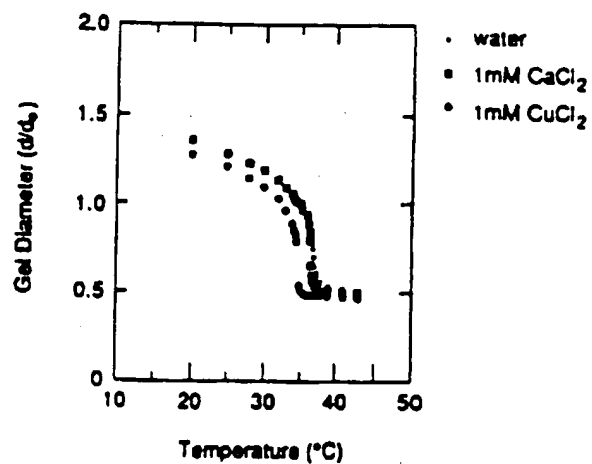


Figure 4

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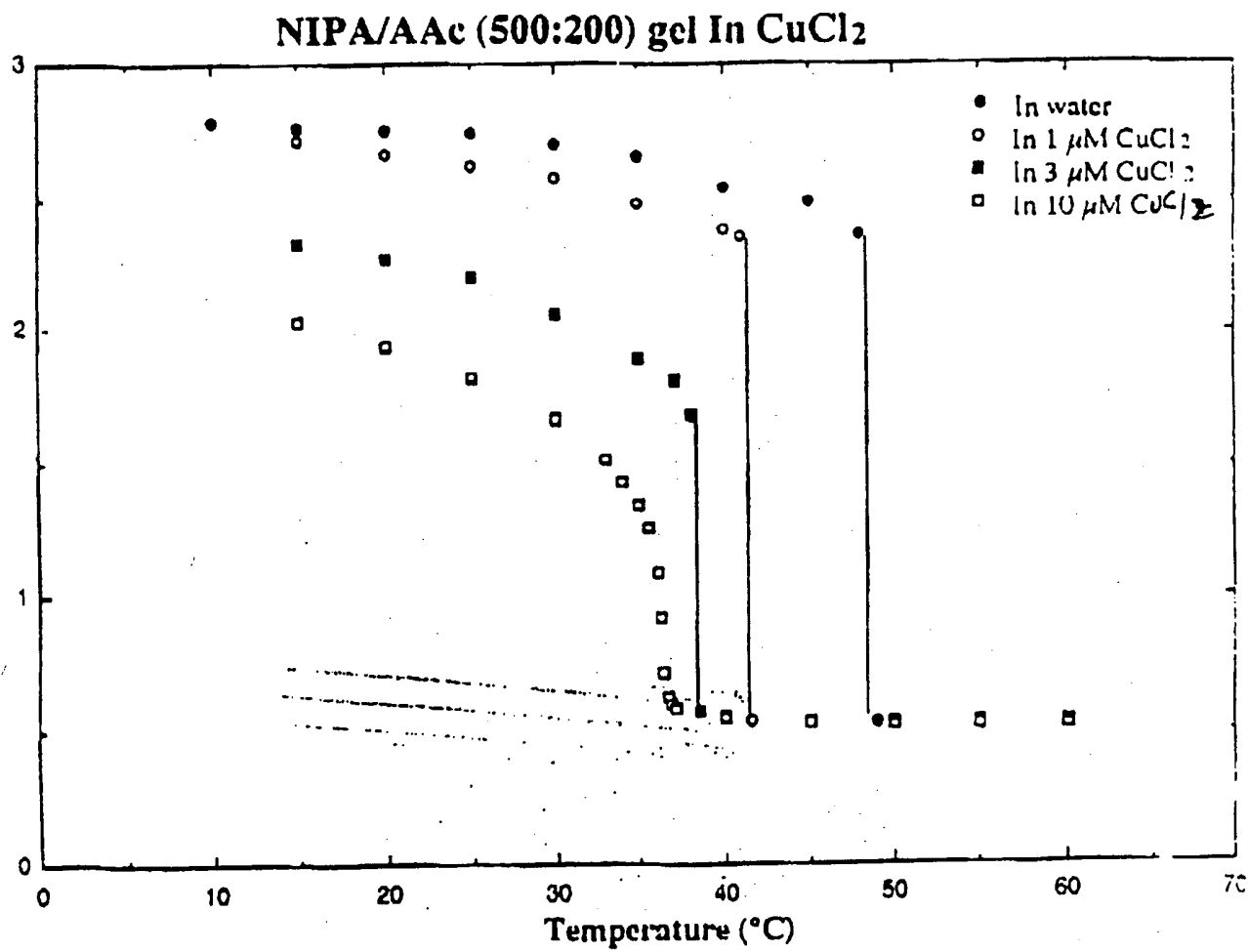


FIGURE 2

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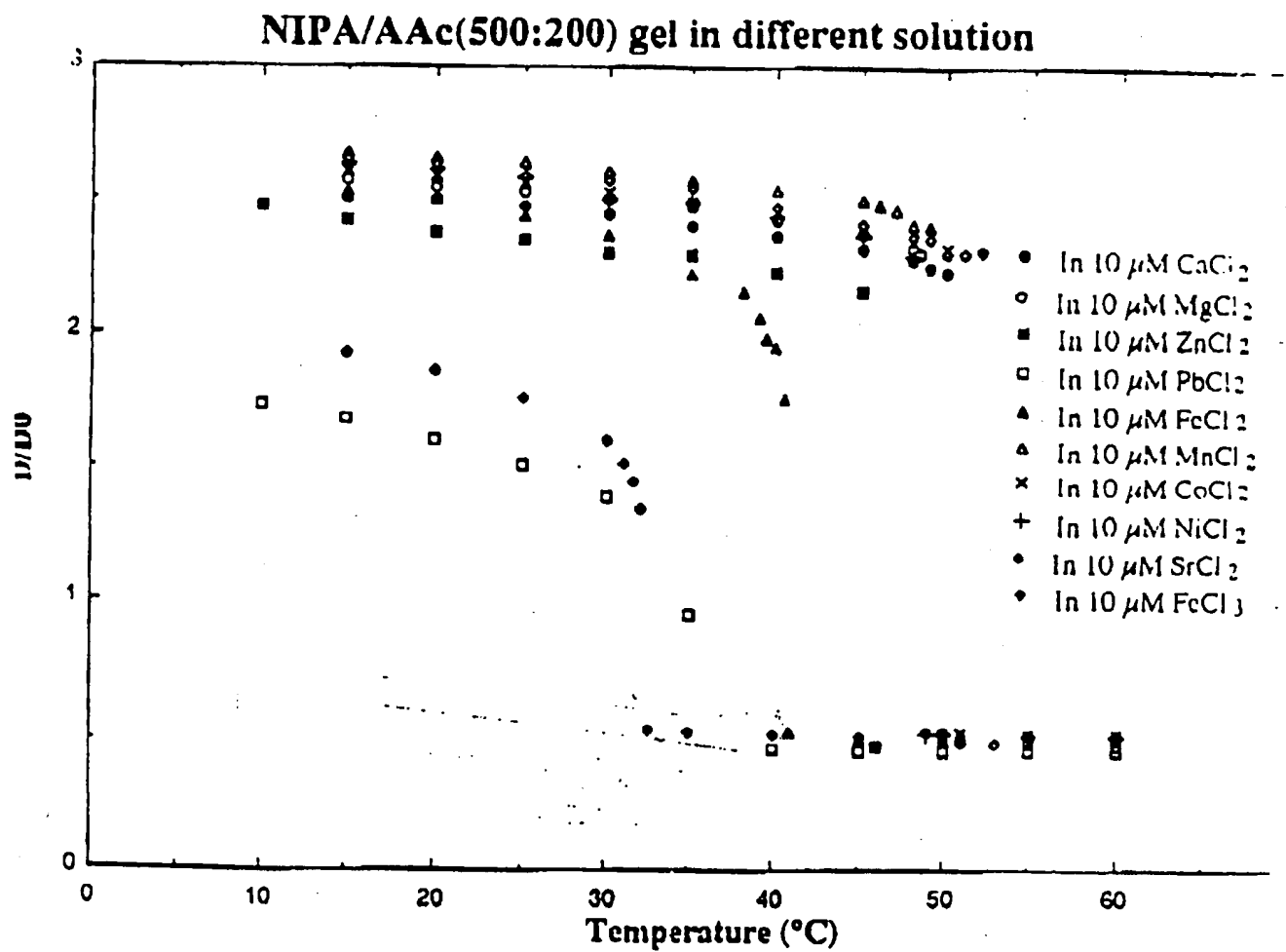


FIGURE 3

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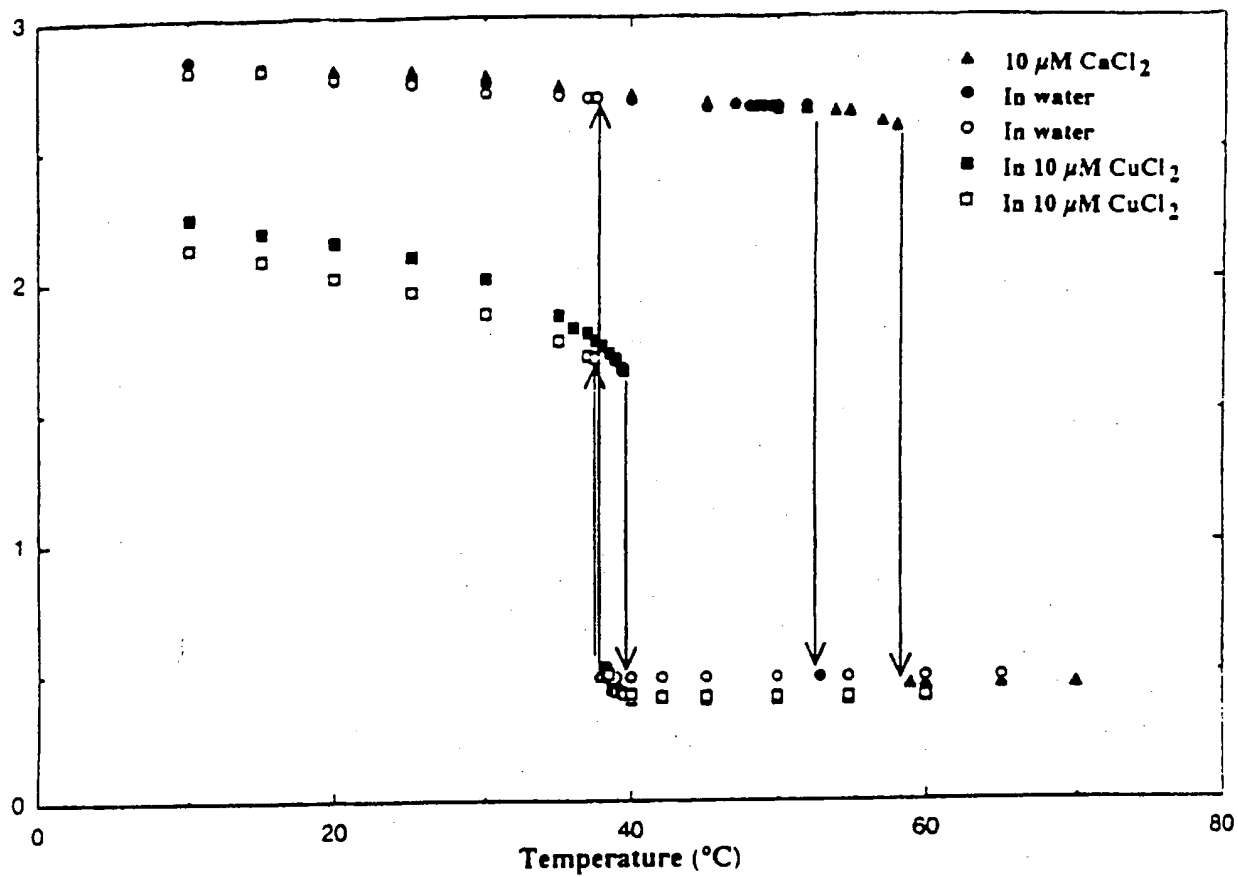
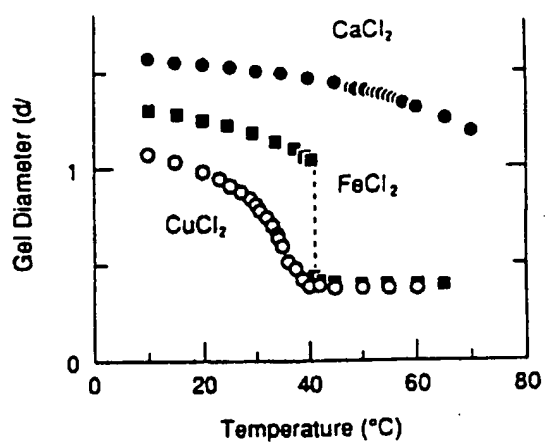


FIGURE 5

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FIG. 6



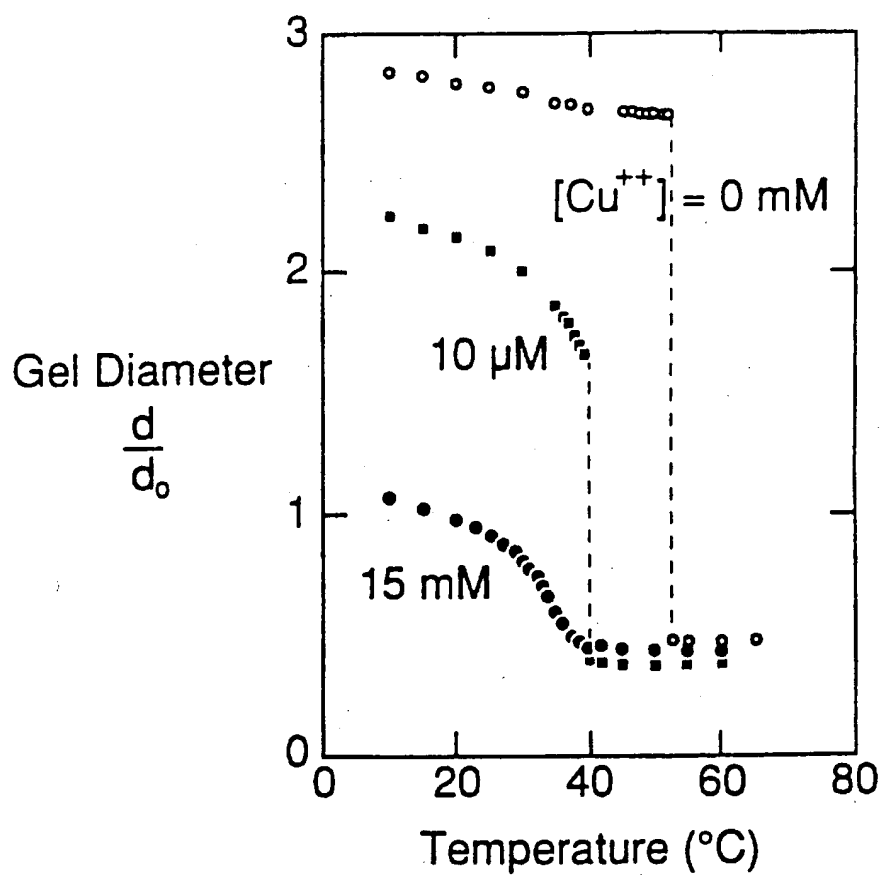


Figure 7 The Phase Transition Temperature of NIPA/AAc (500mM/200mM) Copolymer Gel Shifts as the Copper Ion Concentration is Varied

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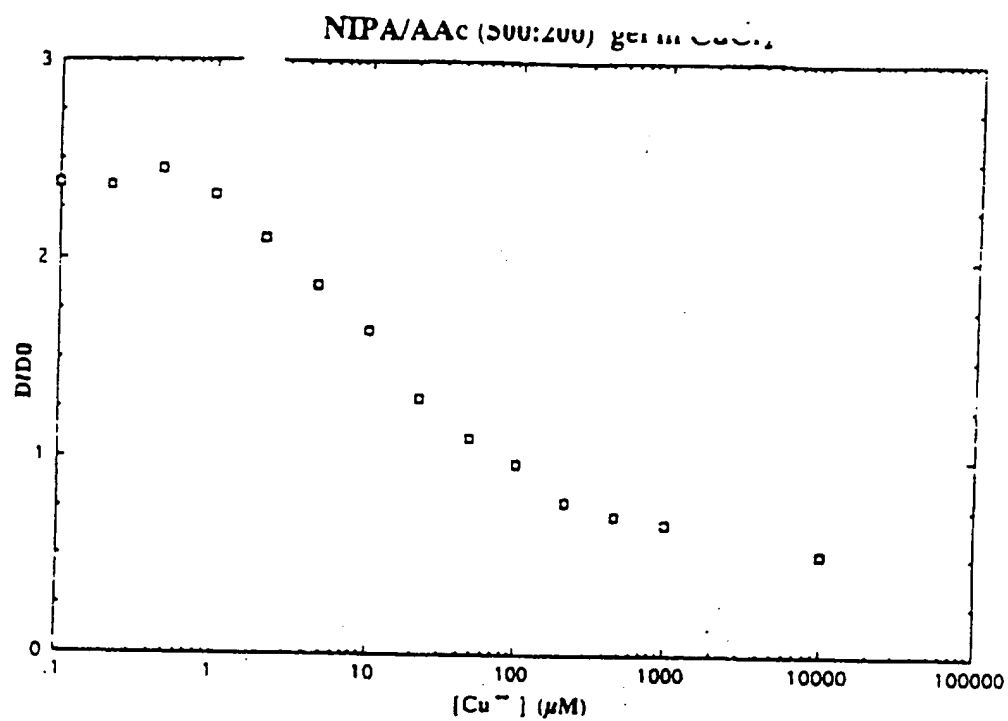


FIGURE 8

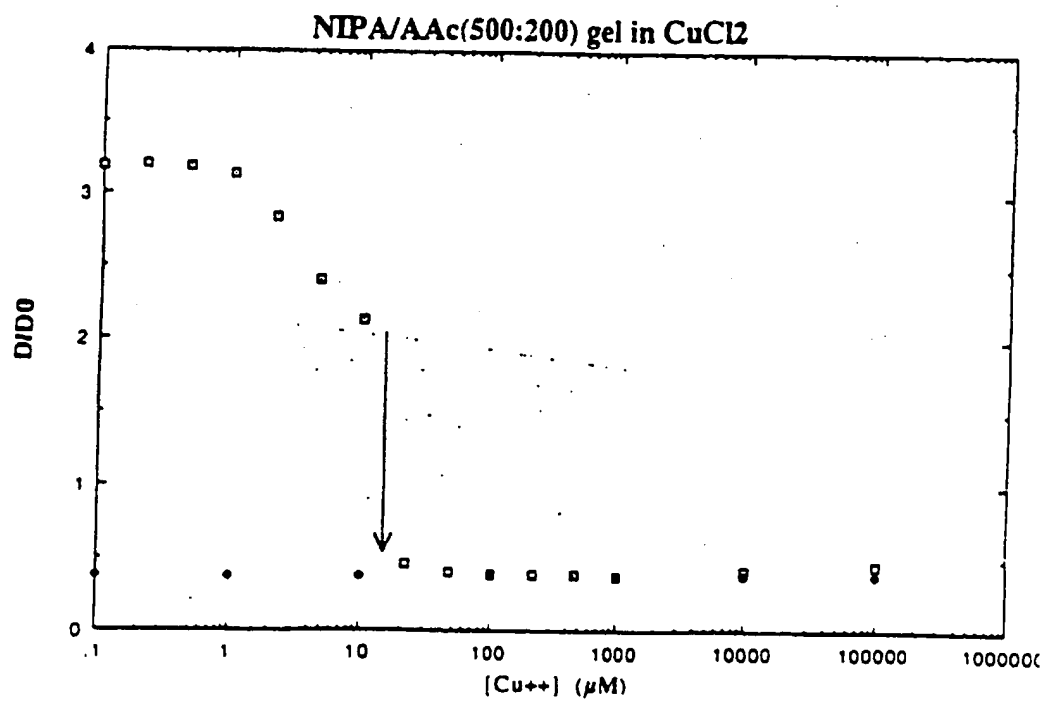


FIGURE 9

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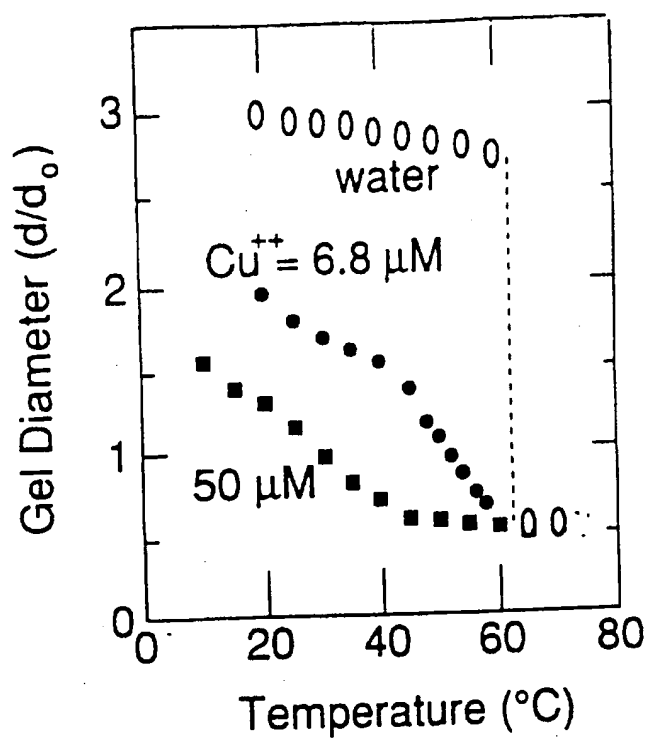


Figure 1C Degree of Swelling of NIPA/AAm/AAc (188mM/256mM/256mM) Copolymer Gel as a Function of Temperature

INTERNATIONAL SEARCH REPORT

Intern

Application No
PCT/US 95/10636

A. CLASSIFICATION OF SUBJECT MATTER

C 08 L 33/24, B 01 J 20/26, C 02 F 1/28

According to International Patent Classification (IPC) or to both national classification and IPC ⁶

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C 08 F, C 08 L, B 01 J, C 02 F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO, A, 92/13 566 (MASSACHUSETTS INSTITUTE OF TECHNOLOGY) 20 August 1992 (20.08.92), page 5, line 25 - page 6, line 23; page 8, line 23 - page 9, line 17; page 14, line 15 - page 19, line 12; exemplification; claims.	1-19
A	US, A, 4 975 375 (HARUTA et al.) 04 December 1990 (04.12.90), column 2, lines 40-59; column 5, line 42 - column 6, line 47; examples 1-3; claims.	1-11

☒ Further documents are listed in the continuation of box C.☐ Patent family members are listed in annex.

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Date of the actual completion of the international search
06 December 1995

Date of mailing of the international search report

22.12.95

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NL - 2280 HV Rijswijk
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INTERNATIONAL SEARCH REPORT

-2-

Intern Application No
PCT/US 95/10636

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>--- PATENT ABSTRACTS OF JAPAN, unexamined applications, C section, vol. 16, no. 411, August 31, 1992 THE PATENT OFFICE JAPANESE GOVERNMENT page 163 C 979; & JP,A,04 139 206 (KOHJIN CO.LTD.).</p>	1-11
A	<p>--- PATENT ABSTRACTS OF JAPAN, unexamined applications, C section, vol. 18, no. 170, March 23, 1994 THE PATENT OFFICE JAPANESE GOVERNMENT page 4 C 1182; & JP,A,05 331 448 (KOHJIN CO.LTD.).</p>	1-11
A	<p>--- US. A, 5 183 879 (YUASA et al.) 02 February 1993 (02.02.93), column 4, lines 24-54; column 6, line 29 - column 7, line 15; examples 1,3,4; claims (cited in the application).</p>	1-19
A	<p>--- US. A, 5 100 933 (TANAKA et al.) 31 March 1992 (31.03.92), column 2, line 37 - column 4, line 56; claims (cited in the application).</p> <p>----</p>	1-19

ANHANG

zum internationalen Recherchen-
bericht über die internationale
Patentanmeldung Nr.

ANNEX

to the International Search
Report to the International Patent
Application No.

ANNEXE

au rapport de recherche inter-
national relatif à la demande de brevet
international n°

PCT/US 95/10636 SAE 116787

In diesem Anhang sind die Mitglieder
der Patentfamilien der im obenge-
nannten internationalen Recherchenbericht
angeführten Patentdokumente angegeben.
Diese Angaben dienen nur zur Unter-
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This Annex lists the patent family
members relating to the patent documents
cited in the above-mentioned inter-
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Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WO A1 9213566	20-08-92	EP A1 569542 JP T2 4506490 US A 5403893	18-11-93 21-07-94 04-04-95
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US A 5100933	31-03-92	US E 35068	17-10-95

